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Structure and properties of pyruvate decarboxylase and site-directed mutagenesis of the Zymomonas mobilis enzyme.

Candy JM, Duggleby RG.

Centre for Protein Structure, Function and Engineering, Department of Biochemistry, University of Queensland, Brisbane 4072, Australia.

Pyruvate decarboxylase (EC 4.1.1.1) is a thiamin diphosphate-dependent enzyme that catalyzes the penultimate step in alcohol fermentation. The enzyme is widely distributed in plants and fungi but is rare in prokaryotes and absent in animals. Here we review its structure and properties with particular emphasis on how site-directed mutagenesis of the enzyme from Zymomonas mobilis has assisted us to understand the function of critical residues.

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Pyruvate decarboxylase of Zymomonas mobilis: isolation, properties, and genetic expression in Escherichia coli.

Neale AD, Scopes RK, Wettenhall RE, Hoogenraad NJ.

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Pyruvate decarboxylase (EC 4.1.1.1) from Zymomonas mobilis purified to homogeneity by using dye-ligand and ion-exchange chromatography. Antibodies produced against the enzyme and the amino-terminal sequence obtained for the pure enzyme were used to select and confirm the identity of a genomic clone encoding the enzyme selected from a genomic library of Z. mobilis DNA cloned into pUC9. The genomic fragment encoding the enzyme expressed high levels of pyruvate decarboxylase in Escherichia coli. Possible RNA polymerase and ribosome-binding sites have been identified in the 5'-untranslated region of the pyruvate decarboxylase gene.

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Nucleotide sequence of the pyruvate decarboxylase gene from Zymomonas mobilis.

Neale AD, Scopes RK, Wettenhall RE, Hoogenraad NJ.

Pyruvate decarboxylase (EC 4.1.1.1), the penultimate enzyme in the alcoholic fermentation pathway of Zymomonas mobilis, converts pyruvate to acetaldehyde and carbon dioxide. The complete nucleotide sequence of the structural gene encoding pyruvate decarboxylase from Zymomonas mobilis has been determined. The coding region is 1704 nucleotides long and encodes a polypeptide of 567 amino acids with a calculated subunit mass of 60,790 daltons. The amino acid sequence was confirmed by comparison with the amino acid sequence of a selection of tryptic fragments of the enzyme. The amino acid composition obtained from the nucleotide sequence is in good agreement with that obtained experimentally.

PMID: 3029726 [PubMed - indexed for MEDLINE]

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Comparison of the structural genes for pyruvate decarboxylase in different Zymomonas mobilis strains.

Reynen M, Sahm H.

Institut fur Biotechnologie, Julich, Federal Republic of Germany.

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The nucleotide sequence of the pyruvate decarboxylase gene from Zymomonas mobilis ATCC 29191 was determined and compared with the sequence of the corresponding gene in Z. mobilis ATCC 31821. Differences were found, leading to variations on the amino acid level and to different sites for restriction endonucleases.

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Cloning and sequencing of the gene coding for alcohol dehydrogenase of Bacillus stearothermophilus and rational shift of the optimum pH.

Sakoda H, Imanaka T.

Department of Biotechnology, Faculty of Engineering, Osaka University, Japan.

Using Bacillus subtilis as a host and pTB524 as a vector plasmid, we cloned the thermostable alcohol dehydrogenase (ADH-T) gene (adhT) from Bacillus stearothermophilus NCA1503 and determined its nucleotide sequence. The deduced amino acid sequence (337 amino acids) was compared with the sequences of ADHs from four different origins. The amino acid residues responsible for the catalytic activity of horse liver ADH had been clarified on the basis of three-dimensional structure. Since those catalytic amino acid residues were fairly conserved in ADH-T and other ADHs, ADH-T was inferred to have basically the same proton release system as horse liver ADH. The putative proton release system of ADH-T was elucidated by introducing point mutations at the catalytic amino acid residues, Cys-38 (cysteine at position 38), Thr-40, and His-43, with site-directed mutagenesis. The mutant enzyme Thr-40-Ser (Thr-40 was replaced by serine) showed a little lower level of activity than wild-type ADH-T did. The result indicates that the OH group of serine instead of threonine can also be used for the catalytic activity. To change the pKa value of the putative system, His-43 was replaced by the more basic amino acid arginine. As a result, the optimum pH of the mutant enzyme His-43-Arg was shifted from 7.8 (wild-type enzyme) to 9.0. His-43-Arg exhibited a higher level of activity than wild-type enzyme at the optimum pH.

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A thermophilic alcohol dehydrogenase from Bacillus acidocaldarius not reactive towards ketones.

D'Auria S, La Cara F, Nazzaro F, Vespa N, Rossi M.

Institute of Protein Biochemistry and Enzymalogy, C.N.R., Naples, Italy.
e021rn02@area.ba.enr.it

An NAD-dependent alcohol-aldehyde oxidoreductase was purified to homogeneity and characterized from cell extracts of the thermophilic microorganism Bacillus acidocaldarius. The 500-fold purified homogeneous enzyme had a molecular mass of 154 kDa, as shown by gel filtration and glycerol gradient centrifugation. On sodium dodecyl sulfate polyacrylamide gel electrophoresis the protein showed one band of 38 kDa, indicating that the enzyme is a tetramer composed of subunits of identical molecular weight. Ethanol was the best substrate with the highest kcat/Km values, and the enzyme showed a substrate specificity that included linear, secondary and cyclic alcohols, as well as anisaldehyde, but it was not active on ketones. The protein contains eight zinc atoms per tetramer, four of which are removed by chelating agents with a concomitant loss of thermal stability. Circular dichroism spectra and determination of the NH2-terminal sequence allowed structural and homology comparison with other alcohol dehydrogenases from animal and bacterial sources.

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Purification and characterisation of the pyruvate decarboxylase from a haploid strain of Saccharomyces cerevisiae.

Killenberg-Jabs M, Konig S, Hohmann S, Hubner G.

Martin-Luther-Universitat Halle-Wittenberg, Fachbereich Biochemie/Biotechnologie, Halle/Saale, Germany.

A novel purification procedure was developed for pyruvate decarboxylase (PDC, E.C. 1.1.1.4) from the haploid yeast strain YSH 4.127-1A expressing only one (PDC1) of the three structural genes for PDC. The purified enzyme is homotetrameric with a molecular mass of about 240,000 whereas PDC from brewer's yeast is a dimer of dimers composed of subunits of different size (alpha 2 beta 2) with the same molecular mass as the tetramer. Despite these structural variations there are no significant differences in the kinetic behaviour of the two enzyme species. PDC purified from the haploid yeast mutants shows a sigmoid dependence of the reaction rate from the substrate concentration due to the substrate activation. In the presence of the substrate surrogate pyruvamide the shape of the v/S plot is transformed into a hyperbolic one. As expected, polyclonal antibodies react with both the enzyme from haploid yeast strain mutants and that from brewer's yeast.

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Aspartate-27 and glutamate-473 are involved in catalysis by Zymomonas mobilis pyruvate decarboxylase.
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The role of residues glutamate-50 and phenylalanine-496 in Zymomonas mobilis pyruvate decarboxylase.
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The replacement of Trp392 by alanine influences the decarboxylase/carboligase activity and stability of pyruvate decarboxylase from Zymomonas mobilis.
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Genetic improvement of Escherichia coli for ethanol production: chromosomal integration of Zymomonas mobilis genes encoding pyruvate decarboxylase and alcohol dehydrogenase II.
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J Bacteriol. 1988 Jul;170(7):3310-3.
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ELSEVIER SCIENCE FULL-TEXT ARTICLE

Purification and characterization of the alcohol dehydrogenase from a novel strain of Bacillus stearothermophilus growing at 70 degrees C.

Guagliardi A, Martino M, Iaccarino I, De Rosa M, Rossi M, Bartolucci S.

Dipartimento di Chimica Organica e Biologica, Universita di Napoli, Italy.

The biocatalysts isolated from thermophilic microorganisms are the object of ever-growing scientific interest for (i) the comprehension of the molecular basis of their thermal tolerance, and (ii) their use in different bio-industrial fields. Here we report the purification and characterization of an alcohol dehydrogenase (designated ADH-hT) from the novel strain LLD-R of Bacillus stearothermophilus which grows at 70 degrees C. ADH-hT was obtained in pure form by anion exchange chromatography and two affinity chromatographies, with a final yield of about 30%. ADH-hT was found to be a tetramer of 37 kDa-subunits, and to have a pI of 4.9. ADH-hT displayed a broad substrate specificity; its activity was highest for aldehydes, and decreased progressively for alcohols and ketones. ADH-hT was endowed with catalytic activity and resistance in the presence of several denaturing agents (organic solvents, detergents, chaotropic agents). ADH-hT shared with ADH 1503 (the alcohol dehydrogenase from B. stearothermophilus strain NCA 1503 which grows at 55 degrees C) the optimal temperature of 65 degrees C, but it was more resistant than ADH 1503 towards heating. In conclusion, due to its stability and broad substrate specificity ADH-hT could be utilized in bio-industrial processes. Furthermore, we believe that ADH-hT could represent a good model system for studying the mechanism(s) which proteins exploit to gain heat resistance.

PMID: 8729010 [PubMed - indexed for MEDLINE]

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Promoter and nucleotide sequences of the Zymomonas mobilis pyruvate decarboxylase.

Conway T, Osman YA, Konnan JI, Hoffmann EM, Ingram LO.

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DNA sequence analysis showed that pyruvate decarboxylase (one of the most abundant proteins in Zymomonas mobilis) contains 559 amino acids. The promoter for the gene encoding pyruvate decarboxylase was not recognized by Escherichia coli, although the cloned gene was expressed at relatively high levels under the control of alternative promoters. The promoter region did not contain sequences which could be identified as being homologous to the generalized promoter structure for E. coli. Hydropathy plots for the amino acid sequence indicated that pyruvate decarboxylase contains a large number of hydrophobic domains which may contribute to the thermal stability of this enzyme.

PMID: 3029037 [PubMed - indexed for MEDLINE]

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